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No benefit from combining HE4 and CA125 as ovarian tumor markers in a clinical setting

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No benefit from combining HE4 and CA125 as ovarian tumor markers in a clinical setting

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ABSTRACT

Objective. About 70% of epithelial ovarian cancer patients (EOC) are diagnosed at advanced stage with a five-year survival rate of only 30%. Whilst CA125 detects peritoneally-spread disease, it has limited sensitivity for early cancers, many of which are potentially curable.

Methods. We compared the new commercially available tumor marker HE4 with CA125 individually, in combination, within the risk of malignancy index (RMI) and the newly defined risk of malignancy algorithm (ROMA). Our prospectively-collected cohort of 160 patients consisted of healthy controls, benign diseases, and borderline tumors/adenocarcinomas of ovarian, tubal, peritoneal and endometrial origin. HE4 and CA125 were measured in serum using standardized ELISA.

Results. Both markers showed similar diagnostic performance in the detection of EOC at clinically defined thresholds (CA125 35 U/ml; HE4 70 pM) but HE4 was not elevated in endometriosis. Comparison of non-malignant diagnoses (n = 71) *versus* early stage ovarian and tubal cancers (n = 19) revealed that HE4 and ROMA displayed the best diagnostic performance (AUC 0.86/0.87, specificity 85.9%/87.3% and sensitivity 78.9%/78.9%, respectively). Whilst RMICA125 detects peritoneal cancer better than all other models (AUC 0.99, specificity 97.2%, sensitivity 80.0%), there is no other detection benefit from RMI compared to HE4 alone or included in ROMA.

Conclusions. The major advantage of HE4 lies in its specificity and improved detection of borderline tumors and early stage ovarian and tubal cancers. HE4 is superior to CA125 with or without RMI and ROMA indices. However, we see no benefit from combining both markers in clinical practice.

Introduction

Since its introduction CA125 has been the only clinically useful tumor marker for the detection of epithelial ovarian cancers (EOC) [1]. At present, the overall five-year survival for patients with EOC is 40% due to 75% of patients being diagnosed as advanced stage disease. In the absence of early detection markers, the risk of malignancy index (RMI) is currently being used in combination with CA125 [2]. Its fast and easy calculation enables gynecologists to triage patients with probable EOC to specialized gynecological oncology centers [3].

As combined tumor markers might detect a larger fraction of early FIGO stage EOC, several efforts have been undertaken to identify adjuncts to CA125 [4–7]. HE4 (Human Epididymis 4) has evolved as a promising marker identified during the genomic era [8] with several studies reporting elevated mRNA expression of *HE4* in various subtypes of EOC [5–7,9–11] prompting investigations on its usefulness as a new tumor marker [10,12–22]. Whilst no study has examined the predictive value of HE4 within RMI, a recent publication has proposed a new risk of malignancy algorithm (ROMA) which combines a logarithmical formula of HE4 levels with the menopausal status [19].

With the increasing clinical availability of HE4, our aim was to define the clinical benefit gained by adding it to the present panel of ovarian tumor markers. We measured the detection efficacy of HE4 compared to CA125 within various risk indices with the aim to identify clear benefits compared to existing clinical alternatives. This is therefore the first study which measured and compared not only

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individual HE4 and CA125 detection rates within various gynecological cancers but also their combination alone and in two clinically available risk models, RMI and ROMA.

Materials and methods

ELISA

Patients admitted to the University Hospital Zurich were prospectively included after giving informed consent in accordance with ethical regulations (SPUK, Canton of Zurich, Switzerland). Patients with a history of cancer or autoimmune diseases were excluded. Three major patient groups were evaluated: 1. proven healthy patients based on normal findings during surgery due to false ultrasonic abnormalities or therapeutic procedures like tubal ligation; 2. abnormalities/benign diseases diagnosed due to pathological CA125 levels or ultrasonic abnormalities like cystadenomas/adenofibromas or endometriosis; and 3. borderline tumors and adenocarcinomas of ovarian, tubal, peritoneal or endometrial origin. All clinicopathological patient data such as FIGO stage, grade, residual disease, presence of ascites, past and present medical illness, ultrasonic findings and outcome data were stored in a specially designed in-house database (PEROV) based on ACCESS (Microsoft, USA). Histopathology of all study patients were independently re-evaluated by a pathologist specialized in the field of gynecological oncology (R.C.), and patients with unclear or mixed diagnoses were excluded from the study.

Blood samples were collected in EDTA blood tubes (BD Vacutainer®, 0184 M EDTA, BD Diagnostics, Franklin Lakes, NJ, USA) prior to surgery and stored on ice until further processed. Samples were centrifuged at 4 °C within 3 h at 3000×*g* for 10 min and supernatant stored at −80 °C. ELISA for HE4 (Prod. Nr. 404–85; Fujirebio Diagnostics Inc., Goteborg, Sweden) and CA125 were performed in a blinded customized fashion (Fujirebio Diagnostics Inc., Goteborg, Sweden).

Statistical analysis

Serum values were calculated in units per ml (U/ml) for CA125 and in picomolar (pM) for HE4 and were additionally incorporated into RMI (RMIHE4, RMICA125). RMI is calculated using the product of serum CA125 level, ultrasound scan result (expressed as a score of 0, 1 or 3) and menopausal status (1, premenopausal and 3, postmenopausal) [2]. An individual tumor marker combination (HE4×CA125) was defined as a product of absolute values of HE4 and CA125. These were further combined by multiplication to standard RMI criteria (RMIHE4×CA125). ROMA calculates the coefficient for the natural log (LN) of serum values and integrates it into a logistic regression formula for pre-menopausal [Predictive Index (PI) = −12.0 + 2.38×LN (HE4) + 0.0626×LN(CA125)] and post menopausal [PI = −8.09 + 1.04×LN(HE4) + 0.732LN(CA125)] women [19].

All data analysis was performed using the open source statistical programming language R (http://CRAN.R-project.org, version 2.8.1). Each binary classifier was analyzed by receiver operating characteristics (ROC; R package ROCR) to determine its area under the curve (AUC), sensitivity (Sens) defined as true positive rate and specificity (Spec) as true negative rate. The clinical thresholds for pathological tumor marker values as defined by the manufacturers were 35 U/ml (CA125) and 70 pM (HE4). A suspicious mass as defined by RMICA125 showed a value of >200 arbitrary units (U) [2] and for HE4 (RMIHE4) of >400 U. The threshold for the combination of both CA125 and HE4 was 2450 U (35 U/ml×70 pM) and within RMI (RMIHE4× CA125) as 14,000 U.

Results

Our cohort consisted of 160 patients divided into three independent sub-groups, (1) thirty-three healthy controls, (2) seventy-one patients with benign diseases, and (3) fifty-six tumor patients. The benign cohort consisted of cystadenomas (n = 32), teratomas (n = 12) and endometriomas (n = 20) whilst the tumor cohort incorporated patients with ovarian and peritoneal borderline tumors (n = 8), endometrial cancers (n = 5), adenocarcinoma of ovarian (n = 29), tubal (n = 6) and peritoneal (n = 5) origin and incidental stromal tumors of the ovary (n = 3) (Table 1, Table 2). Overall, there were slightly more pre- (n = 84) than post-menopausal women in our cohort (n = 76) (Table 2).

Healthy controls and patients with benign diseases had low levels for HE4 of 53 and 50 pM (interquartile range (IR) 39–62; 42–62, respectively) as well as CA125 levels of 7 U/ml both (IR 4–12; 4–13, respectively) (Fig. 1). The cancer cohort had a median concentration of HE4 of 128 pM (IR 79–572) and for CA125 of 62 U/ml (IR 16–304). Advanced peritoneal and tubal cancers had the highest median values for both HE4 (411 pM, IR 193–1450; 703 pM, IR 127–2115, respectively) and CA125 (2280 U/ml, IR 189–6390; 134 U/ml, IR 31–1330, respectively) (Fig. 1). Patients with borderline tumors had a median

Table 1
Clinicopathological characteristics of the patient cohort (n = 160).

Disease status		n	%	Total (%)
Healthy control (33)		33	100	20.6
Benign diseases (71)	Simple cyst	4	5.6	44.4
	Paratubarcyst	3	4.2	
	Cystadenoma/fibroma	32	45.1	
	Endometriosis	10	14.1	
	Endometrioma	10	14.1	
	Teratoma	12	16.9	
Borderline tumors and cancers (56)	Borderline tumor	8	14.3	35.0
	Epithelial ovarian cancer	29	51.8	
	Peritoneal cancer	5	8.9	
	Tubal cancer	6	10.7	
	Endometrium cancer	5	8.9	
	MMMT	2	3.6	
	Stromal sarcoma	1	1.8	
Stage (56)	I	15	26.8	
	II	10	17.8	
	III	21	37.5	
	IV	8	14.3	
	N/A	2	3.6	
Grade (48)	1	5	10.4	
	2	15	31.1	
	3	26	54.2	
	N/A	2	4.3	
Histotype (56)	Serous	26	46.4	
	Endometrioid	13	23.2	
	Mucinous	6	10.7	
	Clear cell	2	3.6	
	Transitional cell	3	5.4	
	Others	6	10.7	

Table 2
Clinical and biochemical characteristics of the patient cohort (n = 160).

		Disease status		Patients (%)
		Non-malignant	Borderline/malignant	
Age (years)	<50	51	10	61 (38.1)
	≥50	53	46	99 (61.9)
Menopausal status	pre	62	22	84 (52.5)
	post	42	34	76 (47.5)
CA125 [U/ml]	<35	96	24	120 (75.0)
	≥35	8	32	44 (25.0)
HE4[pM]	<70	89	13	102 (63.7)
	≥70	15	43	58 (36.3)
ROMA (%)	<13.1	89	11	100 (62.5)
	≥13.1	15	45	60 (37.5)
RMICA125 (U)	<200	101	22	123 (76.9)
	≥200	3	34	37 (23.1)

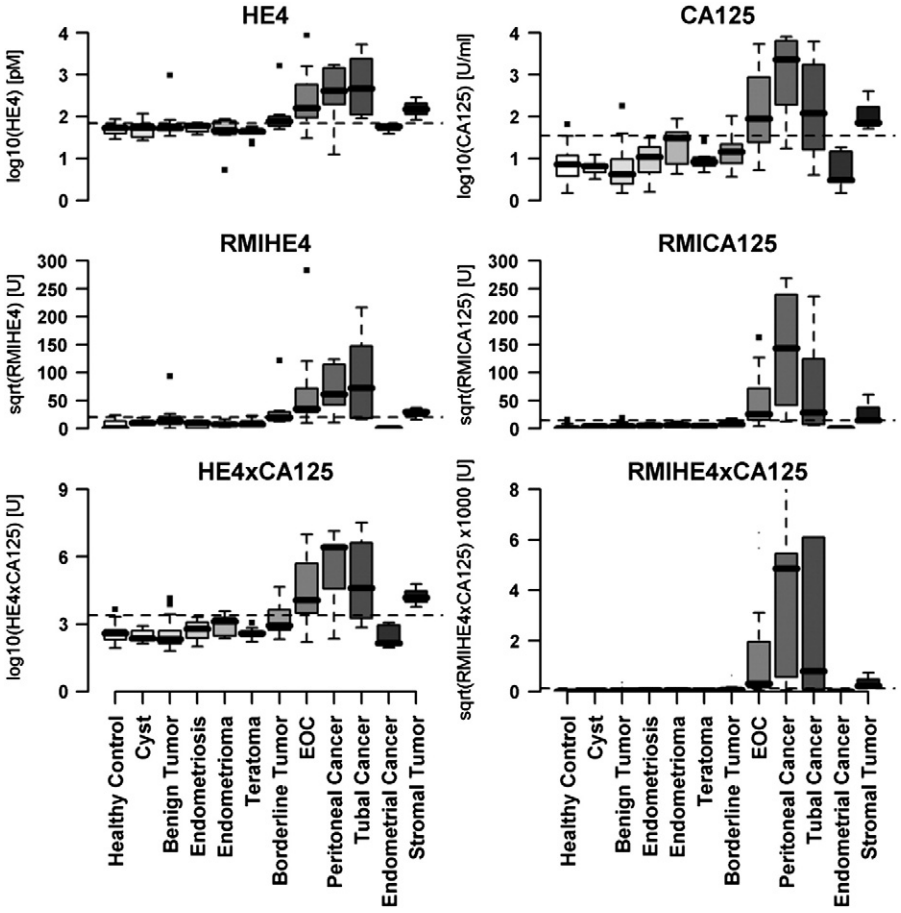

Fig. 1. Marker distribution. Boxplots of HE4 and CA125 levels alone and combined in RMI; black dotted line indicates threshold to separate non-malignant conditions from EOC.

value close to the detection threshold of 77 pM (HE4, IR 59–96) and 14.4 U/ml (CA125, IR 9–20) (Fig. 1). Patients with endometrial cancers did not have abnormal levels of HE4 (58 pM, IR 47–61) or CA125 (3 U/ml, IR 3–14) (Fig. 1) and failed to be detectable with any model indicated by low AUC (range 0.03–0.54), specificity (78.8–97.2%) and sensitivity (0–40.0%). Endometrial cancers were therefore not included in further specific comparisons.

ROC analysis of individual tumor markers and their combinations were performed for the whole patient cohort (non-malignant (n = 104) *versus* malignant (n = 48)) and revealed similar high AUC values for all calculated models (range 0.87–0.95). HE4 performed best (Spec 84.6%; Sens 83.3) with only a slightly better result within ROMA (Spec 85.6%; Sens 85.4) (Table 3). HE4 and ROMA showed an improved specificity when the borderline/cancer cohort was compared to benign diseases (85.9%/87.3%, respectively) instead of healthy controls (81.8%/78.8%, respectively). We also compared endometriosis (n = 20) to the borderline/cancer group (n = 48) which revealed the best performance using ROMA (AUC 0.89,

Table 3
Receiver operator curves on selected biomarker models. Area under the curve (AUC), specificity (Spec, in %), sensitivity (Sens, in %) revealed by comparison of healthy controls, benign diseases, endometriosis (EM) or whole non-malignant group *versus* borderline tumors (BL), epithelial ovarian (EOC), peritoneal (PC), tubal (TC) and endometrial cancers (EC). Threshold: HE4 70 pM; CA125 35 U/ml; HE4*CA125 2450 U; ROMA 13.1%; RMIHE4 400 U; RMICA125 200 U; RMIHE4*CA125 14,000 U.

Versus		HE4			CA125			CA125*HE4			ROMA			RMIHE4			RMICA125			RMICA125*HE4		
		AUC	Spec	Sens	AUC	Spec	Sens	AUC	Spec	Sens	AUC	Spec	Sens	AUC	Spec	Sens	AUC	Spec	Sens	AUC	Spec	Sens
Healthy controls	EOC	0.92	81.8	86.2	0.92	93.9	67	0.95	97	82.8	0.92	81.8	89.6	0.97	93.9	89.6	0.97	93.9	75.9	0.98	100	86.2
	BL	0.81		62.5	0.72		12.5	0.79	93.9	25	0.79	78.8	62.5	0.89		50	0.91		37.5	0.92	97	37.5
	PC	0.80		80	0.97		80	0.87		80	0.82		80	0.92		80	0.99		80	0.97		80
	TC	1.00		100	0.85		66.7	0.95		66.7	0.98		100	0.97		66.7	0.85	100	50	0.97		66.7
	EC	0.53		0	0.39		0	0.38		0	0.44		40	0.25		0	0.26	93.9	0	0.25		0
	I/II + BL	0.86		78.9	0.79		36.8	0.85		52.6	0.85		78.9	0.92		63.1	0.94		47.4	0.95	100	63.2
	III/IV	0.93		89.3	0.95		78.6	0.96	97	85.7	0.94	81.8	92.8	0.98		92.8	0.98		82.1	0.98	97	85.7
Benign disease	EOC	0.91	85.9	86.2	0.89	90.1	69	0.93	92.9	82.8	0.93	87.3	89.6	0.94	83.1	89.6	0.96	98.6	75.7	0.96	97.2	86.2
	BL	0.83		62.5	0.67		12.5	0.76		25	0.83		62.5	0.83		50	0.82	97.2	25	0.86		37.5
	PC	0.80		80	0.96		80	0.86		80	0.82		80	0.89		80	0.99		80	0.94	95.8	80
	TC	0.99		100	0.83		66.7	0.93		66.7	0.98		100	0.93		66.7	0.92		66.7	0.97		66.7
	EC	0.54		0	0.38		0	0.37		0	0.45		40	0.03		0	0.03		0	0.03		0
	I/II + BL	0.86		78.9	0.76		36.8	0.83		52.6	0.87		78.9	0.87		63.2	0.89		47.4	0.90	97.2	63.2
	III/IV	0.93		89.3	0.94		78.6	0.95		85.7	0.94		92.8	0.95		92.8	0.98	98.6	82.1	0.98		89.3
EM	Cancer + BL	0.89	80	83.3	0.76	80	60.4	0.84	90	71	0.89	80	85.4	0.96	100	79.2	0.92	100	66.7	0.93	100	75
Non-malignant	Cancer + BL	0.89	84.6	83.3	0.87	91.3	60.4	0.90	94.2	70.8	0.90	85.6	85.4	0.93	86.5	79.1	0.95	97.1	66.7	0.95	98.1	75

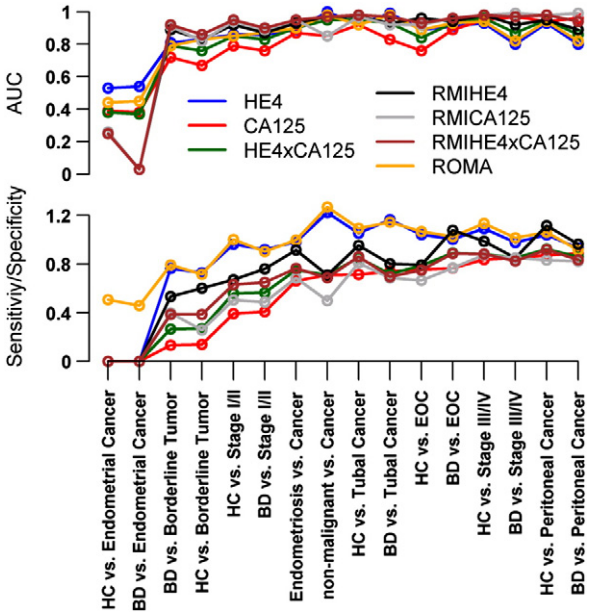

Fig. 2. Summarized ROC values. AUC and ratio of sensitivity divided by specificity summarized for each binary classifier. Each colored line presents ROC for an individual model. Comparisons sorted by the ratio sensitivity/specificity of CA125 (red line). Ratio of 1 where sensitivity and specificity are equal (gray line); healthy control (HC); benign disease (BD).

specificity 80.0% and sensitivity 85.4%) followed by HE4 with equal AUC value and specificity but less sensitivity (83.3%) (Table 3).

Borderline tumors were best detected using HE4 alone compared to the group of healthy control or benign disease with a AUC of 0.81/0.83, specificities of 81.8%/85.9% and sensitivities of 62.5%/62.5%, respectively (Fig. 2). ROMA performed similar to HE4 with AUC of 0.79/0.83, specificities of 78.8%/87.3% and sensitivities of 62.5%/62.5%. The lowest sensitivity in the detection of borderline tumors from healthy controls or patients with benign diseases could be found for CA125 (AUC 0.72/0.67, specificity 93.9%/90.1%, sensitivity 12.5%/12.5%).

All models revealed the same sensitivity of 80.0% for comparison of healthy controls/benign diseases and peritoneal cancers but RMI-CA125*HE4 detected it best (AUC 0.97/0.94, specificity 97.0%/95.8%; Fig. 2). HE4 and ROMA revealed here the worst performance with AUC of 0.80/0.82 and specificity reduced by about 10%–15% compared to CA125 alone (Table 3).

The ability to detect early cancers would definitely improve patient prognosis. Therefore, we studied HE4 and CA125 detection rates for FIGO Stage I and II borderline tumors (3 mucinous, 4 serous and ovarian/tubal cancers of serous (n = 4), endometrioid (n = 4), mucinous (n = 3) and transitional cell (n = 1) subtypes. The best detection compared to healthy controls (n = 33) or benign diseases (n = 71) was achieved with ROMA (AUC 0.85/0.87, respectively) and HE4 (AUC 0.86/0.86, respectively) (Table 3).

Conclusion

Emerging from the genomic era, HE is the most prominent and second commercially available tumor marker for EOC. Recent studies observed a better diagnostic performance of HE4 compared to CA125 (Table 4), however, it is difficult to directly compare these publications because the detection threshold has been variable and cancer sensitivity and specificity was measured either in relation to benign diseases or healthy controls (Table 4). We ensured that only individuals with proven negative operative findings were included as healthy controls to exclude cases of asymptomatic endometriosis or pelvic inflammatory disease. Indeed we were able to show that healthy controls and benign diseases differ slightly and if pooled together can influence the diagnostic performance rate in comparison to cancers.

Our data support an advantage of HE4 over CA125 mainly in the detection of ovarian borderline tumors and early stage epithelial ovarian [19] and tubal cancers. The similar expression profile of both markers accounts for the finding that additional risk indices have not improved the detection rate more than a few percent. The combination of both markers (HE4*CA125, ROMA, RMIHE4*CA125) does not improve the diagnostic performance of HE4 alone and does not overcome the inability of both markers to adequately detect early stage epithelial ovarian cancers, with or without RMI or ROMA. The only potential benefit we see from a combination of CA125 with HE4 would be in a premenopausal woman with a high RMI due to an elevated CA125. A normal HE4 value in this situation would imply rather the differential diagnosis of a benign endometrioma [19] than ovarian cancer and this patient could therefore be operated laparoscopically by a gynecologist.

If there would be a conjoint decision to use HE4 as new tumor marker for ovarian, tubal and peritoneal cancers and was similarly available, it would have clear benefits for the detection of these diseases. However, as long as CA125 is still widely used, the urge to simultaneously measure both markers will cause an unnecessary rise in costs for minimal benefits. Currently, HE4 is available for double the price of CA125, but the combination of both markers will triple the costs. Moreover, screening measurements of tumor markers in nonsymptomatic women is clearly not justified and has not proven any benefit in early detection, let alone by a combination of markers involving CA19-9, CEA and CA72-4 to include mucinous tumors. Testing for both HE4 and CA125, alone or combined with any clinical risk assessment, is therefore not advisable for clinical routine.

Conflict of interest statement
This work was partially funded by Fujirebio Diagnostics, Goteborg, Sweden (VHS). None of the other authors declare a conflict of interest.

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Table 4
Publications investigating HE4 as tumor marker compared to CA125. Epithelial ovarian (EOC) and endometrial cancers (EC), borderline tumors (BL), healthy controls (HC).

Study	Pre-defined threshold	Comparison	CA125	HE4	Others	
			Sensitivity/specificity in percentage (%)			
Current study	CA125 (35 U/ml)	Non-malignant vs. cancer	60.4/91.3	83.3/84.6	85.4/85.6	66.7/97.1
	HE4 (70 pM)	HC vs. stage I/II + BL	36.8/93.6	78.9/81.8	78.9/78.8	47.4/93.9
	RMI125 (200 U)	Benign vs. stage I/II + BL	36.8/90.1	78.9/85.9	78.9/87.3	47.4/97.2
		Endometriosis vs. cancer BL	60.4/80.0	83.3/80.0	85.4/80.0	66.7/100
	ROMA (13.1%)				ROMA	RMICA125
Nolen et al. [21]	Upper 95th percentile of the benign groups	Benign vs. EOC (training)	79.5/85.0	70.5/85.0	83.0/85.0	
		Benign vs. EOC (validation)	76.3/82.1	83.4/84.3	89.4/77.9	
		Benign vs. EOC (premeno)	70.7/87.5	43.1/93.8	62.1/87.5	
		–Multiplex bead-based assay–			CA125–HE4	
Abdel-Azeez et al. [12]	CA125 (35 U/ml)	Benign vs. EOC	73.2/79.2	82.9/87.5	79.2/90.2	
		Benign vs. early stage EOC	61.5/79.2	76.9/87.5	79.2/84.6	
	HE4 (72 pM)				CA125–HE4	
Moore et al. [19]	Specificity of 75%	Benign vs. EOC/OBL			80.7/75.0	89.0/75.0
		Benign vs. EOC			84.6/75.0	94.3/75.0
		Benign vs. early stage EOC	n/a	n/a	64.7/75.0	85.3/75.0
		Benign vs. late stage EOC			93.0/75.0	98.8/75.0
					RMI	ROMA
Montagnana et al. [16]	CA125 (37 U/ml); HE4 (30 pmol/l)	Healthy vs. EOC	83/100	98/100	n/a	
Shah et al. [22]	Specificity of 95%	Healthy average risk vs. EOC	79.4/95.0	80.4/95.0	53.9/95.0	
		Healthy high risk vs. EOC	82.9/95.0	87.8/95.0	39.0/95.0	
		Benign average risk vs. EOC	58.8/95.0	61.8/95.0	43.1/95.0	
		Benign high risk vs. EOC	63.4/95.0	75.6/95.0	34.1/95.0	
					Mesothelin	
Huhtinen et al. [15]	Specificity of 95%	Endometriosis vs. EOC	64.3/95.0	71.4/95.0	78.6/95.0	
		Healthy vs. EOC	78.6/95.0	78.6/95.0	92.9/95.0	
		Endometriosis vs. healthy	60.9/95.0	05.8/95.0	62.3/95.0	
					CA125 + HE4	
Moore et al. [20]	Predictive probability (PP) threshold 13.1%	Benign vs. cancer[a]	n/a	n/a	88.7/74.7	
		Benign vs. cancer[a] (premeno)			76.5/74.8	
		Benign vs. cancer[a] (postmeno)			92.3/74.7	
					ROMA	
Andersen et al. [13]	Upper 95th percentile of the benign groups	Healthy vs. EOC	81.1/94.9	77.0/94.6	63.5/88.3	
		Healthy vs. EOC (<50 years)	83.3/90.9	83.3/100	83.3/90.0	
		Healthy vs. EOC (>50 years)	81.4/96.3	76.3/93.6	57.6/89.0	
					Symptom Index (SI)	
Moore et al. [17]	Specificity of 95%	Healthy vs. EC	24.6/95.0	45.5/95.0	50.1/95.0	
		Healthy vs. stage I EC	20.8/95.0	37.9/95.0	41.7/95.0	
Moore et al. [18]	Specificity of 95%	Benign vs. EOC	43.3/95.0	72.9/95.0	76.4/95.0 (CA125 + HE4)	
					79.1/95.0 (CA125 + HE4 + CA72-4 + mesothelin + osteopontin)	
Havrilesky et al. [14]	Best cutoff (BC); mean + 2SD (2SD)	Healthy vs. early stage EOC (BC)	45.9/98.2	82.7/86.3	80.5/96.5	
		Healthy vs. late stage EOC (BC)	58.5/98.2	92.5/86.3	89.2/97.2	
		Healthy vs. early stage EOC (2SD)	45.9/98.5	62.4/96.0	CA125 + HE4 + Glyco-delin +	
		Healthy vs. late stage EOC (2SD)	58.5/98.5	74.6/96.0	Plau-R + MUC1 + PAI-1 (BC)	
Hellstrom et al. [10]	Specificity of 96%	Benign/healthy vs. EOC	40.0/96.0	67.0/96.0	n/a	
		Benign/healthy vs. early stage EOC	71.0/96.0	86.0/96.0		
		Benign/healthy vs. late stage EOC	80.0/96.0	80.0/96.0		

[a] Defined as EOC, OBL, non-EOC, metastatic and other gynecological cancers.

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